enzyme. For Y639F, k_{cat} values for reactions with different dNTPs were only 2-4 fold less than for rNTPs, while the w.t. enzyme displayed k_{cat} values with dNTPs that were from ~6 to ~30-fold less than for rNTPs.

Elongation rates of Y639F in reactions containing a single dNTP: Elongation rates for Y639F in reactions with 4 rNTPs or 1 dNTP and 3 rNTPs were determined by analyzing aliquots, taken at 10 second intervals, from transcription reactions initiated on supercoiled PT75 by adding NTPs to otherwise complete reaction mixes (Fig. 6).

Fig. 6 shows relative elongation rates of Y639F in "4 rNTP" and "3 rNTP + 1 dNTP" reactions. The template was supercoiled pT75 at 5×10^{-7} M. Y639F polymerase was used at a concentration of 10^{-6} M. Reactions contained the indicated NTPs. Labeling was with α-P^{32}-rATP (lane e) or α-P^{32}-rGTP (other lanes). After initiation of the reactions aliquots were taken at 10 second intervals and analyzed on 1% agarose denaturing-formaldehyde gels. The figure shows the 20 second time point. The bars indicate the positions of λ DNA markers.

When analyzed on denaturing agarose gels (Fig. 6) the heterogeneously sized transcripts from these reactions are resolved as a smear with the trailing edge of the smear corresponding to transcripts initiated at t=0 and from which the maximal transcript elongation rate can be determined (Golomb and Chamberlin, 1974; Bonner, et al., 1994). The following elongation rate reductions (relative to a '4 rNTP' reaction) were obtained for reactions containing a 3 rNTPS and 1 dNTP:dATP, ~3-fold; dUTP, ~2-fold; dGTP ~1.5-fold; dCTP, 1-1.5-fold. Because of its poor activity in reactions with dNTPs we could not determine the corresponding elongation rate reductions for the w.t. enzyme.

Other non-canonical nucleoside triphosphate substrates for mutant polymerases: Although wild-type T7 RNAP can not efficiently utilize dideoxy-NTPs as substrates, we have found that Y639 mutants of this enzyme can also use dideoxy-NTPs as substrates (Table VII). We have also found that Y639 mutants can use other non-canonical nucleoside triphosphates as substrates (Table VIII). Nucleic acids synthesized by incorporation of some non-canonical nucleotides, such as 2'-F-NTPs, may offer advantages in being more resistant to digestion by nucleases such as ribonucleases. Other uses and advantages of various non-canonical nucleotide substrates in methods of the present invention using the mutant polymerases will be apparent after examination of the specification, claims and drawings.

Other mutations: It is conceivable that, in the absence of a bound rNTP, a hydrogen bond forms between Y639 and some other active site side chain. The possibility of an interaction between M635 and Y639 was tested since M635 and Y639 are close and M635 approaches the ribose in our models of NTP in T7 RNAP (Huang, et al., submitted for publication). This position is methionine in the T7 RNAP class of RNAPs (McAllister, W.T., 1993), but is either tyrosine or phenylalanine in the homologous DNAPs, and in the DNAP mutants at this site (i.e., positions homologous to position 762 in *E. coli* DNAP I) that affect dNTP/ddNTP discrimination (Tabor. S., and Richardson, C.C., European patent application, 1994). While M635A, M635F or M635Y mutants had effects on NTP K_m, they did not affect 2'-group discrimination with respect to dNTPs and rNTPs in either wild-type or the Y639F T7 RNAP background. Additional studies will reveal whether these M635 mutations have other effects, such as effects on discrimination at the 3'-

position of the sugar, or effects on discrimination with respect to NTPs with other substituents, such as fluorine at the 2′ position of the sugar. Also, studies on similar double mutations at the homologous sites in the homologous class I DNAPs will reveal the effects of such double mutations on discrimination at the 2′- and 3′-positions of the sugar; specifically, these studies will reveal whether class I DNAPs having a phenylalanine at the position homologous to amino acid position 766 in *E. coli* DNAP I have reduced discrimination for rNTPs if the amino acid is methionine or tyrosine at the position homologous to amino acid position 762 in *E. coli* DNAP I.

Provided that class I DNAP mutants which have a reduced discrimination for rNTPs compared to dNTPs can be obtained, it will be possible to use such DNAP mutants to carry out the methods of the present invention that comprise nucleic acid synthesis from a nucleic acid primer, at least part of which is sufficiently complementary to a template nucleic acid to hybridize therewith and to be extended by the polymerase. An especially preferable use for such mutant DNA polymerases would be to carry out Partial Ribo-substitution sequencing reactions from primers, whether labelled by any of the methods known in the art, or unlabelled. Since the sequence-delimiting rNTP nucleotides for the Partial Ribo-substitution Reaction do not terminate the growing phosphodiester chain when they are incorporated during nucleic acid synthesis, the DNA synthesis for Partial Ribo-substitution can occur simultaneous with and be identical to nucleic acid synthesis for another procedure, such as NASBA, 3SR, TMA or another similar method, provided that the mutant DNAP with reduced discrimination for rNTP compared to dNTPs is thermostable, or the PCR strand